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In Vitro Fertilization and Embryo Transfer: A Brief Overview

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The *in vitro* fertilization process breaks down into three essential components: induction of ovulation, fertilization of the oocyte, and development of embryos that are transferred into the uterus. Problems may arise resulting in failure at any one of these junctions.

In 1984, the World Congress on In Vitro Fertilization was held, looking at 9,641 laparoscopies yielding 1,101 clinical pregnancies, with an overall pregnancy rate of 11 percent—clearly indicating that *in vitro* fertilization/embryo transfer (IVF/ET) was an idea whose time had come.

Ovulation induction is monitored by both the use of ultrasound and daily estradiol levels, ultrasound indicating the number of oocytes that will be available for capture, and estradiol indicating in an indirect way the quality of those oocytes. It is a major aim in each patient to obtain at least four embryos, since this optimizes success rates. Ovulation induction at Yale is carried out with a high-dose human menopausal gonadotropin (HMG)/human chorionic gonadotropin (HCG) regimen. This regimen has insured us a success rate of 17 percent clinical pregnancies per laparoscopy.

In the future, modifications will occur in the process with cryopreservation of oocytes and embryos, and gamete manipulation. The modifications will be effected primarily to increase pregnancy rates. Research will continue mainly to delineate better biochemical markers for oocyte quality, but also to further explain the mystery of implantation.

INTRODUCTION

In 1984, a World Conference was held on *in vitro* fertilization. Dr. Seppala of the University of Helsinki collected data from most of the centers performing IVF in the world at that time. He reviewed 9,641 laparoscopies, yielding 1,101 clinical pregnancies; an overall pregnancy rate per laparoscopy of 11 percent. The percentage of males was 50.4 percent [1]. Because of the size of the sample, this is certainly the reference point against which all other IVF programs must be measured (Table 1).

The *in vitro* fertilization (IVF) process breaks down into three essential technical components: *(1)* the induction of ovulation in order to get numerous oocytes, *(2)* the fertilization of the oocytes and the development of early embryos in the laboratory, and *(3)* the transfer of these embryos back into the uterus. Areas for potential failure in the system parallel these components. Failure of ovulation can occur, in that either no oocytes or oocytes which will not fertilize are produced; once successful induction has occurred, oocytes must be retrieved at the optimal time for fertilization. Another area for concern is the failure of normal events to occur in the laboratory, including fusion of the sperm to the oocyte membrane, failure of the sperm to penetrate into the ooplasm, failure of the nuclei of the egg and sperm to fuse, and failure of cleavage of the embryo to occur. Problems may arise associated with transfer and implantation. Since so little

TABLE 1
Type of Infertility and Success Rate [1]

Type of Infertility	No. of Pregnancies/No. of Cycles	Success (%) per Cycle
Primary	239/3,051	7.8[a]
Secondary	226/1,965	11.5[a]
Tubal factor	539/5,056	10.7
Endometriosis	46/562	8.2
Unknown cause	74/847	8.7

[a] $p < 0.001$ Published with permission [1]

is known about implantation in general, very few cogent conclusions can be drawn. The aim of the *in vitro* fertilization/embryo transfer process is to mimic nature as best possible within an artificial system.

INDUCTION OF OVULATION AND RETRIEVAL OF OOCYTES

The first portion of the process involves induction of ovulation. The problems dictated include getting a number of oocytes so that a number of embryos can be replaced, since many centers have shown that the pregnancy rate is directly proportional to the number of embryos replaced, up to the number four [2,3]. In conjunction with this is insurance that the oocytes are at a level mature enough to be fertilized and not too old so as to make them degenerated. This is a delicate balance to achieve. The mode of ovulation induction used at Yale is human menopausal gonadotropin (HMG; Pergonal, Serono Laboratories) starting on day 3 through day 8; other combinations of drugs for ovulation induction have been used, including clomiphene, clomiphene + HMG, and natural cycles alone, without an apparent change in success rates [1]. In some of these instances, the luteinizing hormone (LH) surge is used as the trigger point to determine when the patient should have an ovum recovery, rather than the exogenous trigger which, in most cases, is human chorionic gonadotropin (HCG). Pergonal is a combination of 75 mIU follicle-stimulating hormone (FSH) and 75 mIU LH. Then, starting on day 8, daily ultrasounds are done, and once on ultrasound two follicles >1.5 cm are found, 10,000 mIU HCG is given, which stimulates ovulation and ovum maturation [4]. This is done in conjunction with estradiol/E_2 values; if these values are less than 400 pg/ml the cycle is aborted because of supposed poor follicle quality. Another confounding variable at this point is that many follicles are at different levels of generation, i.e., dysynchrony, so one can see a corpus luteum in an ovary coexisting with immature follicles. This stage is dependent on the concept of recruitment of different cohorts of follicles during a single stimulated cycle.

A major goal must be one of ovum quality, since supposedly the better the ovum, the better the embryo, and therefore the higher the pregnancy rate. Many attempts have been made to ascertain ovum quality, but it appears that estrogen production by the granulosa cells of the follicles seems to be the best marker to date [5,6]. High basal levels of E_2 which begin to surge with HCG correlate well with the pregnancy rates in the Yale system. When comparing patients with the surge, the pregnancy rate is 15 percent per laparoscopy as compared to patients without the surge, whose pregnancy rate is 3 percent. Preliminary work using pure FSH to induce ovulation shows promise of minor increases in success rates [7].

Once ovulation induction has been completed and a number of follicles that are at the preovulatory phase have been generated, the next juncture, technically, is ovum

capture. The classic mode of ovum capture is in the operating room where, under general anesthesia using laparoscopy, the follicles are perforated with a needle, transabdominally aspirated, and taken to a nearby laboratory for evaluation. The other major technique for ovum capture is aspiration which exclusively uses either transabdominal or transvaginal ultrasound. The former is done through the bladder under ultrasonic visualization. The ovum recovery rate is slightly less for both ultrasound groups, but pregnancy rates are essentially the same [8]. A subset of patients is defined in which the adhesive disease is so extensive that the ultrasonographer or the laparoscopist is unable to capture any ovum because the ovary is encapsulated in dense adhesions. These patients usually require a pre-IVF/ET laparotomy in order to suspend their ovaries, thereby making them accessible for future ovum capture [9].

OOCYTE FERTILIZATION IN THE LABORATORY

Once the ova are collected, they are taken to the laboratory where, under the dissecting microscope, the oocytes are counted and graded. Grading is based primarily on the cumulus mass and its dispersion. The more dispersed the cumulus mass, the more mature the oocyte, thus facilitating penetration by spermatozoa.

Laboratories maintain various methods of culture, but the World Collaborative Study seems to indicate that HAM-F-10 is the most common media utilized [1]. Once the oocytes are graded, they are either inseminated after six hours or immature oocytes are allowed to mature over a 24-hour period before being exposed to spermatozoa. This discovery, that oocytes can mature *in vitro,* was a major breakthrough since it increased the number of fertilizable oocytes by a factor, in some instances, of as much as 20 percent [10,11].

The spermatozoa are prepared in most labs by a swim-up technique where the sperm is spun down in a centrifuge and allowed to swim up into a supernatant layer of media. Supernatant containing sperm is then placed in a dish or test-tube vial in conjunction with the oocyte; usually between 100,000 and 500,000 spermatozoa per oocyte are utilized. The number of spermatozoa per oocyte, which was apparently critical at one time, seems to be of little significance over 50,000. It is not true that the greater number of spermatozoa the oocyte is exposed to, the more likely is polyspermia to occur. Polyspermia means that more than one sperm penetrates the oocyte. Polyspermia occurs in approximately 16 percent of oocytes, but not all in the same patient [12]. IVF/ET now holds promise for some infertile males, in that men with low sperm counts are now able to fertilize oocytes using IVF with some reproducibility. The World Collaborative Study showed that although pregnancy rates were lower in men with low sperm counts, it was not zero (Table 2) [1].

Much work has gone on over the past six years looking at follicular fluid in order to find markers within this readily available substance that will predict ovum quality, which is essential for fertility and embryo cleavage [6]. Cyclic AMP represents an interesting aspect of this investigation since it probably influences oocyte maturity inhibition; therefore, the lower the cyclic AMP levels in follicular fluid, the more likely is that oocyte to be mature and therefore the more likely it is to become an embryo [13].

EMBRYO TRANSFER AND LUTEAL PHASE

After 24 hours of incubation, the oocytes are then observed for fertilization and cleavage and, approximately 20 hours after this, embryo transfer is performed. Extensive work has been done in looking at the optimal time for embryo transfer; to

TABLE 2
Quality of Semen and Success Rate in IVF [1]

Quality	No. of Pregnancies/No. of Cycles	Success (%) per Cycle
Normal	401/4,136	9.7[a]
Abnormal	60/872	6.9[a]

[a] $p < 0.025$ Published with permission [1]

date there is no answer to this question. The transfer process is simply done by placing a silastic catheter into the uterine cavity through the internal os of the cervix, either with or without a metal introducer. The embryos are then layered into the uterine cavity and, it is hoped, implantation occurs. Very little is known about implantation; some work has looked at two factors in regard to the endometrium, i.e., endometrial receptivity and embryo-endometrial synchrony. This stage still remains an area of great mystery for most clinicians working in the field of *in vitro* fertilization and embryo transfer. One aspect that has been looked at in regard to factors enhancing implantation is supplementation of the luteal phase with progesterone. Progesterone injections seem to be the most common, with other groups using various other regimens for luteal support, including progesterone suppositories and continuous HCG injections [1].

IVF/ET OUTCOME

The evaluation of results remains paramount in importance, both for making advances in the technology and for quality control of centers performing IVF/ET. When looking at normal pregnancy rates if one looks at 100 ovulatory cycles, 60 of those will show some evidence of fertilization, based on very sensitive radioimmunoassays for β-HCG. Of these 60, 25 will turn out to be clinical pregnancies, and three of these conceptions will turn out to be spontaneous abortions [14]. One must consider, then, not only the clinical pregnancy rate but the viable pregnancy rate, as well. In the World Collaborative Study, the viable pregnancy rate was 15 percent with a 29 percent abortion rate [1]. In our own series, we had a 17 percent pregnancy rate per laparoscopy, with a 4 percent abortion rate [15]. Certainly, the next questions that must be evaluated are what is the pregnancy rate per patient, not per cycle, and how many cycles are optimum for a patient to try IVF/ET.

A major concern of society and science originally was the chromosomal integrity of these babies conceived *in vitro*. The World Collaborative Study, as compiled, puts to rest most of these concerns in that the incidence of fetal anomalies and chromosomal abnormalities seems to be, at the most, equal to if not less than the norm [1].

Problems in this area would result in the observation that any of the prior events had not occurred, or it could be demonstrated by chromosomal abnormalities. The rate of chromosomal damage, as far as offspring are concerned, is small (Table 3).

CONCLUSIONS

In conclusion, IVF/ET represents a new technology that is quite exciting for the reproductive endocrinologist and biologist [16]. The technological advances dictate a number of challenges, including ovulation with an aim toward synchronization of oocytes and normalization of oocytes. It dictates management of the external milieu in order to optimize pregnancy rates. An example of this could be the harmful effect of

TABLE 3
Chromosome Anomalies in Spontaneous Abortions After IVF [1]

No.	Type
1	69, XXY
2	47, XX, + C
3	4 Trisomy
4	47, XY, + 4
5	12 Trisomy
6	2 Trisomy
7	Triploid, malformations
8	Triploid
9	21 Trisomy

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the high estrogen levels necessitated by ovulation induction on the endometrium for implantation. Much will be learned about implantation in the future, and perhaps this is the next area of active research after oocyte quality parameters have been relatively exhausted. Close vigilance as to results and careful attention to quality control are paramount to any successful program.

Future considerations for IVF/ET include the use of cryopreservation. Cryopreservation has the advantages of allowing us to utilize only four embryos per cycle, and use the "spare" embryos in order to be replaced in subsequent cycles. This technique has three advantages: *(1)* decreasing the number of laparoscopies needed by the patient; *(2)* doing embryo transfers in a non-stimulated cycle, which therefore means that the embryo does not see the abnormal hormonal milieu that we have created; and *(3)* decreasing, it is hoped, the number of multiple gestations that we achieve now by putting all embryos back. Another area of active research is further delineating biochemical markers of oocyte quality. Eventually, gamete and embryo manipulation will be done in order to optimize success rates.

An example of such manipulation would be to take an embryo, split the embryo, and therefore implant two embryos from a single embryo, creating an identical twin gestation. This microcosmic problem has multiple ethical considerations, which have to be, and are, dealt with on a daily basis in regard to any IVF/ET program.

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